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Research Article

Optimising eDNA analysis for urban otter monitoring: seasonal patterns, detection strategies and prey availability

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Abstract

Significant advancements in environmental DNA (eDNA) analysis technology have led to its widespread adoption for species monitoring. However, the low DNA concentration in eDNA often raises concerns about PCR bias, which is a primary issue in enhancing the reliability of eDNA survey results. This study aims to identify the suitable eDNA analysis method and detection strategy for obtaining the presence data of Eurasian otters in an urban stream and to investigate the seasonal distribution patterns of Eurasian otters and their potential prey species. The eDNA survey revealed the presence of six out of the 13 mammal species recorded in fieldwork and literature reviews, including *Canis lupus*, *Felis silvestris* and *Mustela sibirica*. Notably, Eurasian otters were not detected in the eDNA metabarcoding conducted in April and October, despite traditional surveys confirming their presence. In contrast, qPCR assays successfully amplified Eurasian otters from the same samples that were analysed using metabarcoding. When evaluating detection criteria, based on the number of positive samples in repetitions, Eurasian otters were detected at 42.9% to 85.7% of the sampling sites in April and at all sampling sites in October. This suggests a higher detection probability of Eurasian otters in October, indicating a potential expansion of their home range during that season compared to April. Metabarcoding results revealed similar findings regarding fish species as traditional surveys, with Cyprinidae accounting for the largest proportion of fish species at the family level (April, 54.57%; October, 43.58%), followed by Gobiidae (April, 16.90%; October, 22.88%). At the species level, *P. parva* was the dominant fish species in Saetgang, constituting 5.68% and 6.35% of relative abundance in April and October, respectively. This implies that Eurasian otters as opportunistic predators, may increasingly take advantage of the availability of species within the family Cyprinidae, notably *Pseudorasbora parv*a, as a food source within the study area during the months of April and October. This study highlights that qPCR is the more effective approach in urban areas to offer insights into otters' distribution patterns, while metabarcoding is useful to provide the properties of the biological environment. Furthermore, this study indicated that it is necessary to determine the suitable eDNA analysis methods depending on the research purpose to obtain detection results effectively.

Key words: Environmental DNA, Eurasian otter, metabarcoding, targeted PCR, urban stream

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Introduction

Environmental DNA (eDNA) analysis technology has advanced considerably in recent years, leading to the widespread use of eDNA surveys for species monitoring. This conservative investigation method involves detecting genetic material released by organisms (such as faeces, skin, hair and eggs) and is characterised by a brief analysis process and high sensitivity (Bohmann et al. 2014). Next-generation sequencing enables the detection of multiple species and determination of species composition in environmental samples (Valentini et al. 2016). Alternatively, targeted PCR using conventional or quantitative PCR (qPCR) with a set of species-specific primers can effectively identify a single species (Davy et al. 2015; Hernandez et al. 2020). Previous studies have shown that eDNA results can provide not only presence-absence data, but also quantitative data, given the positive proportion correlation between eDNA amplification and the biomass/population size of species (Takahara et al. 2012; Di Muri et al. 2020; Breton et al. 2022). This approach has been utilised to describe distribution patterns and population sequential changes in several studies, especially for rare and low-density species, such as endangered and invasive species (Thomsen et al. 2012; Buxton et al. 2018; Zhang et al. 2019). Several studies have suggested that eDNA investigations can be used to understand the ecological characteristics of species, such as distribution patterns, potential habitat estimation and food resource analysis. The discovery of species through eDNA is not limited to a specific taxonomic group, but has also been used to detect various species, such as mammals, birds, reptiles and amphibians (Miya et al. 2015; Ishige et al. 2017; Ushio et al. 2018). For example, Buxton et al. (2018) evaluated the seasonal changes in the eDNA concentration of *Triturus cristatus* and discovered that the concentration of eDNA reflected the breeding season and larval abundance. Yamamoto et al. (2017) used eDNA metabarcoding to define fish community structures in species-rich coastal areas. Furthermore, eDNA detection results have been applied as input data to model settings, such as species distribution models (SDMs) and occupancy models for evaluation and prediction (Muha et al. 2017; Da Silva Neto et al. 2020; Riaz et al. 2020).

On the one hand, eDNA has a low concentration of DNA, so improving accuracy and reproducibility has consistently been required. PCR bias is usually mentioned as the main issue that needs to be controlled to increase the reliability of eDNA survey results (Smith and Peay 2014; Krehenwinkel et al. 2017; Mauvisseau et al. 2019; Shirazi et al. 2021). For instance, when using eDNA metabarcoding to detect terrestrial animals in a water body, the 'species masking effect' can occur, whereby high-abundance species like fish prevent the detection of low-abundance species like terrestrial mammals (Lintermans 2016; Yu et al. 2022). Therefore, targeted PCR for single species detection could be more effective in enhancing the detection probability of fewer individuals or lower DNA concentration. However, targeted PCR with low-concentration samples can produce different presence-absence assessments of target species depending on the number of PCR repetitions in the laboratory or sampling repetitions at study sites. Consequently, it is important to determine the differences in detection results, especially for low-abundance species, by experimental conditions and target species and to select the appropriate methodology according to the purpose of the survey.

The Eurasian otter (*Lutra lutra*) is an endangered species distributed widely from Europe to Asia (Loy et al. 2022). It is a carnivorous animal that feeds on fish, birds and other aquatic organisms and plays a crucial role in evaluating the stability of ecosystems as an umbrella species (Erlinge 1967). With the increase in food sources due to urban stream restoration projects and water quality management, Eurasian otters have expanded their habitat to urban areas in Korea. As a semi-aquatic mammal that inhabits water bodies and has low density in certain areas, it has been selected as a model species to compare the detection probability between metabarcoding and qPCR using water samples (Harper et al. 2019a; Macher et al. 2021; Mariani et al. 2021; Ritter et al. 2022). Previous investigations have employed metabarcoding and qPCR methodologies to explore the distribution and dietary habits of Eurasian otters (Harper et al. 2020; Jang-Liaw 2021; Jamwal et al. 2023). Additionally, species diversity has been assessed through the analysis of spraints metabarcoding (Dou et al. 2023). The research question of this study is to determine the appropriate eDNA analysis method and detection strategy to obtain the presence data of Eurasian otters. Subsequently, based on the eDNA metabarcoding and qPCR results, we aim to investigate the distribution pattern of Eurasian otters and potential food species by season.

Materials and methods

Study area and sampling design

eDNA surveys and trace-tracking fieldwork were conducted in Yeouido Saetgang Ecological Park, which is in Seoul, South Korea. Saetgang is a small river that branches off from the Han River and flows around an alluvial island called 'Yeouido'. The Yeouido Saetgang Ecological Park is a green space that emphasises ecological elements, such as emergent plant communities, eco-friendly ponds and bioswales, with a total length of 4.6 km and a total area of 758,000 m^2. To identify the typical distribution of the Eurasian otter in Saetgang, both surveys were conducted from the river entrance to the confluence points. Along the river, six sampling sites were located at intervals of approximately 500 m (YS1, YS2, YS4, YS5, YS6 and YS7). Additionally, one sampling site was in a pond connected to the river inside the Ecological Park (YS3) (Fig. 1). To assess the physical environment at each study site, we examined stream width, actual water width and substrate composition. The substrate was assessed in five classes, based on particle size: B (Boulder, > 256 mm), C (Cobble, 64–256 mm), P (Pebble, 4–64 mm), G (Gravel, 2–4 mm) and S (Silt, < 2 mm) (Suppl. material 3).

Sampling: eDNA and traditional field survey

The eDNA sampling at seven sites in study area was conducted on 27 April (spring) and 11 October 2022 (autumn). To compile an adequate species list for the study area, we used Sterivex cartridge filters with a 0.45 µm pore size (Millipore), assuming replication and filtered 330 ml through each filter for a total of 990 ml of water (Sato et al. 2017; Dickie et al. 2018). In addition, we filtered the water in the vivarium of Eurasian otters at Korean otter Research Center as the positive eDNA sample. Contamination occurring during the

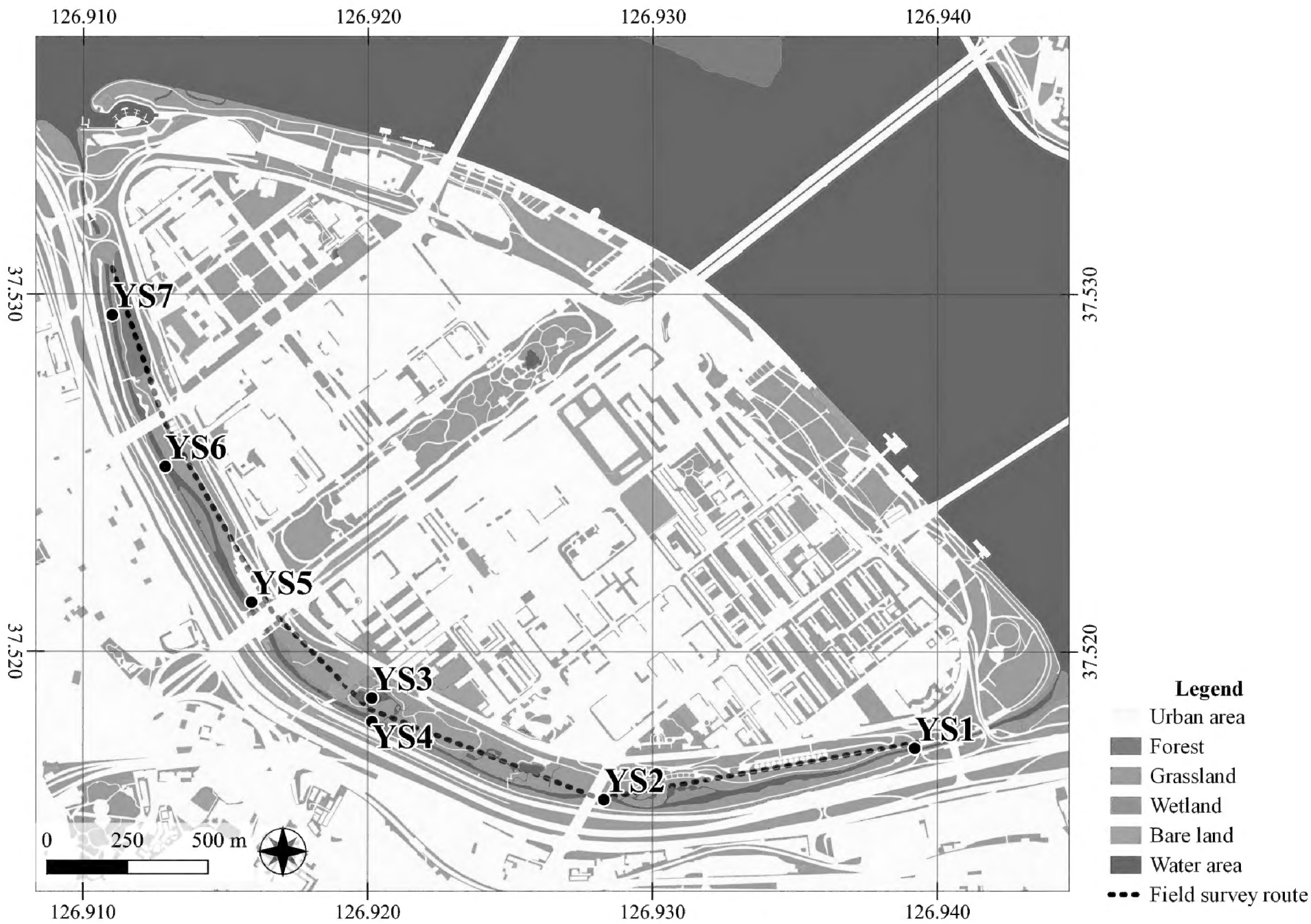

Figure 1. The location of seven sampling sites in Yeouido Saetgang Ecological Park.

sampling process was monitored using negative controls. Independent sampling materials including cartridge filter and 30 ml syringe were used to prevent contamination. After filtration, cartridge filters were stored in an ice box to prevent degradation and later moved to the laboratory and kept at -20 °C in a refrigerator before extraction. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Germany). Extracted DNA was stored at -20 °C and quantified using a Nanodrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA) before sequencing.

To enhance the reliability of the environmental DNA survey findings, simultaneous observational surveys for fish and mammals were conducted. The traditional field survey, an indirect survey to identify mammalian habitats and traces of movement, was carried out by professionals in pairs on 21 July (summer) and 18 October (autumn) 2022. Summer and autumn were chosen as the survey periods to accurately ascertain the presence of otters in the target area, given that these seasons correspond to their low and high activity phases in South Korea, respectively (Kim 2018; Jo and Won 2019; Lee et al. 2020). Traces such as tunnels, footprints, dead bodies, food foraging, scat, ground nest and hair were investigated along the Saetgang. During the same period, fish were surveyed using a kick net, collecting fish for 40 minutes at each survey point and a casting net was employed 10 times. At the collection site, fish were identified to the species level, based on their morphological traits using Korean reference books (Kim 1997; Kim and Park 2002; Kim et al. 2005) and then released. This

survey method is routinely utilised in the national natural environment survey in Korea (National Institute of Environmental Research 2016) In addition, a literature review preceded the fieldwork to determine the past presence of the Eurasian otter and other mammals in Yeouido between 2002 and 2019 (Gang et al. 2015; Seoul Metropolitan Government 2017; Yeongdeungpo Cultural Foundation 2020).

Metabarcoding of vertebrate species

To determine vertebrate species and their taxon, DNA amplification was used together with three universal primers called MiFish (Miya et al. 2015), MiBird (Ushio et al. 2018) and MiMammal (Ushio et al. 2017). In order to create the Next Generation Sequencing (NGS) library, two phases of PCR were carried out. The first PCR was conducted with a total reaction volume of 12 µl, comprising 2 µl of DNA template, 6 µl of 2× KAPA HiFi ready mix (KAPA Biosystems, Inc., Wilmington, MA, USA), 0.36 µl of forward universal primer and 0.36 µl of reverse primer, with the remaining volume of 3.28 µl filled with distilled water. The three universal primers (MiFish, MiMammal, MiBird) were individually utilised in PCR reaction at a concentration of 10 µM. The primary PCR conditions were set to 35 cycles of 98 °C for 20 seconds of denaturation, 65 °C for 15 seconds of annealing, 72 °C for 15 seconds of extension and 72 °C for 5 minutes. PCR products were visualised on a 1% agarose gel to evaluate amplification success. Prior to the second PCR, the PCR product was purified using 20 µl of Ampure XP Beads. After purification, the concentration of the product was measured using the Infinite F200 PRO (Tecan) and normalised to 20 ng/µl. The reaction mixture for the second PCR was prepared with a total volume of 12 µl, consisting of 6 µl of 2× KAPA HiFi Ready Mix, 3 µl of distilled water, 1 µl of each unique dual index ID (P5 [Nextera, S5, 1 µM] and P7 [Nextera, N7XX, 1 µM], totalling 2 µl) and 1 µl of the normalised DNA template. The cycling conditions were identical to those of the first PCR, except for reducing the cycle number to 12. Following the second PCR, the concentration was measured again using the Infinite F200 PRO and adjusted to 100 ng/µl for sequencing. The Illumina libraries were sequenced using a MiSeq Reagent Kit v.3 (600-cycles) on an Illumina MiSeq 300PE platform (Illumina, San Diego, CA, USA). To address potential contamination, any amplification observed in negative controls may warrant exclusion of the detected organisms or the establishment of a threshold to filter out low-level noise (Forstchen 2020). Additionally, increased sequencing depth enhances the detection of rare species, with sequencing depths between 50,000 and 200,000 reads generally considered appropriate for metagenomic analyses (Bruce et al. 2021). Therefore, in this study, MiSeq sequencing results yielding fewer than 50,000 reads were considered negative, indicating a lack of amplification (Suppl. material 4).

The MiFish pipeline's biogenetic information DB and the FASTQ file it analysed were compared (http://mitofish.aori.u-tokyo.ac.jp/mifish) to create a species list (Sato et al. 2018). For the bioinformatic processing of FASTQ files generated using MiBird and MiMammal, the PMiFish bioinformatics pipeline (PMiFish v. 2.4; the latest version is available from https://github.com/rogotoh/PMiFish.git; Miya et al. 2020) was utilised. Data preprocessing and

analysis of MiSeq raw reads are performed using USEARCH v.10.0.240 (Edgar and Bateman 2010). The process begins with the assembly of quality-filtered forward (R1) and reverse (R2) reads. Subsequently, a length filter is applied to remove primer sequences and retain only those sequences that are not shorter than 140 bp. This is followed by quality filtering to further refine sequence integrity. After quality filtering, sequences undergo dereplication, where a depth filter is applied to ensure that only sequences with at least four replicates are retained. The next steps include denoising, to reduce error rates and taxonomic assignment of the cleaned and verified sequences. Results are rejected for sequences that are ambiguous or chimeric, with a BLASTn similarity of less than 97% to the clustered sequence.

Species list derived from metabarcoding results were refined in consideration of the study area (Gang et al. 2015; Seoul Metropolitan Government 2017; Yeongdeungpo Cultural Foundation 2020). The saltwater fish, alien species undocumented in South Korea and species extinct within native natural ecosystems were omitted from the detected species list (National Institute of Biological Resources 2021). Besides, considering the environment of the target site, some mammal detection results that do not display subspecies, such as wild boars (*Sus scrofa*) and wolves (*Canis lupus*), were recorded as domestic pigs (*Sus scrofa domesticus*) and dogs (*Canis lupus familiaris*), including subspecies. To ensure the accurate estimation of potential food species, we conducted a comparison between the species abundance, quantified by the number of individuals, obtained from traditional surveys and the species abundance measured in terms of the number of reads through eDNA metabarcoding. Before the analysis, the number of eDNA reads underwent a natural logarithmic transformation to standardise the data, consistent with the methodology employed in prior studies (Yates et al. 2019, 2021; Rourke et al. 2022).

Quantitative PCR of target species *L. lutra*

Primer validation

To amplify eDNA of *L. lutra*, otter-specific primers reported by (Park et al. 2011) were used. A set of primers, LutcytF, ‘CCACAATCCTCAACAACTCGC’ and LutcytR, ‘CTCCGTTTGGGTGTATGTATCG’, were designed to amplify a 227 bp long amplicon of the mitochondrial cytochrome b. These reference primers can amplify eDNA of *L. lutra* by conventional PCR using otter tissue and positive eDNA samples collected in a vivarium of *L. lutra* and at study sites in this study. The amplification conditions for validation included an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds and 72 °C for 30 seconds, with a final extension at 72 °C for 7 minutes. As the reaction mix, i-StarTaq™ DNA Polymerase (iNtRON Biotechnology, South Korea) was used for conventional PCR. The reaction mix used i-StarTaq™ DNA Polymerase (iNtRON Biotechnology, South Korea) for conventional PCR. The composition of the reaction mix is as follows: per sample, 0.5 µl of forward primer and 0.5 µl of reverse primer (10 pM), 1 µl of 10× buffer, 1 µl of dNTPs, 0.1 µl of i-StarTaq™ DNA polymerase and 5.9 µl of distilled water (Suppl. material 1).

Detection thresholds and quantification

Quantitative real time PCR (qPCR) assays were conducted with an Quantstudio 3 (ThermoFisher) instrument. qPCR conditions consisted in an initial denaturation at 95 °C for 10 minutes, followed by 35 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds and 72 °C for 30 seconds and melt curve stage for standard curve. For eDNA sample assay, the cycle was increased to 40 under the same PCR conditions. TOPreal™ SYBR Green qPCR PreMIX (Enzynomix) was used for reaction mix. In accordance with the total capacity of 10 µl, it was composed of TOPreal SYBR Green qPCR PreMIX 5 µl, DNA 2 µl, LutcytF 0.5 µl and LutcytR 0.5 µl per sample. Template DNA was 1 µl for generating the standard curve and 2 µl for the eDNA assay. For the negative control samples, an additional 2 µl of ultrapure water was added in place of the DNA template to monitor for contamination. To generate a standard curve, a 10-fold serial dilution was performed from 90 ng/µl of genomic DNA of otter and the assay was repeated in triplicate. The appropriateness of the primer efficiency and standard curve was assessed by ensuring that the efficiency percentage fell between 90 and 110% (Raymaekers et al. 2009). The Limit of Detection (LoD) was calculated using the following equation: where σ represents the standard deviation intercept and s denotes the absolute value of the slope:

$$LoD = [3.3 * (\sigma/s)]$$

A positive sample was determined, based on the LoD and the eDNA concentration was calculated through the standard curve function. Positive eDNA samples were also assessed by threshold criteria. ‘Without Threshold (WT)’ refers to a case where a positive is confirmed in one or more of sampling and qPCR repetition. ‘Repetition Thresholds (RT)’ means determining a positive sample when detecting more than three times out of 10 repetitions of qPCR. ‘Sample Thresholds (ST)’ are evaluated as positive samples when detected more than twice out of three repeated samples. In the absence of any threshold criterion, the lowest reliability is assumed and, in the case of applying the threshold criterion at the sample and repetition level, the highest reliability is assumed.

$$Ct = Slope * \log_{10}(Quantity) + Y\ intercept$$

$$Quantity = 10^{(Ct - Yintercept)/Slope}$$

Results

Field survey results based on observation and literature review

A total of eight families and 13 species of mammals were recorded in the field survey and literature review (Table 1). From 2008 to 2019, there were no records of Eurasian otters in the study area. However, footprints of the Eurasian otter were observed twice during fieldwork for this study in October 2022. Given that the known home ranges of female and male otters are 7 km and 15 km, respectively

Table 1. The mammal species list identified from field survey and literature review in the study area.

Scientific name	Fieldwork in this study (2022)						Literature review				
	1st fieldwork (Jul)			2nd fieldwork (Oct)			2008	2012	2015	2017	2019
	V	F	S	V	F	S					
Canidae											
Canis lupus familiaris	4	3	1	2	3	1		◎			
Nyctereutes procyonoides					1						
Cervidae											
Hydropotes inermis										◎	
Erinaceidae											
Erinaceidae sp.										◎	
Felidae											
Felis silvestris		4		1	3		◎		◎	◎	◎
Muridae											
Apodemus agrarius										◎	
Mus musculus					2		◎				
Rattus rattus							◎				
Rattus norvegicus								◎	◎	◎	
Mustelidae											
Lutra lutra					2						
Mustela sibirica							◎				
Soricidae											
Crocidura lasiura							◎				
Talpidae											
Mogera wogur							◎				

* V, eyewitness; F, footprint; S, scat.

(Néill et al. 2009), it is suggested that the otter can be detected across the study area, which spans 4.5 km. Additionally, mammals commonly found adjacent to urban areas, such as raccoon dogs, feral cats and rodents which were detected as the study sites, are within an urban area. Our observations and literature review did not reveal any presence of wild boars in the study area.

In our field study for fish, we identified a total of four families and 16 different fish species (Table 2). Fish abundance was notably higher at specific locations, particularly at the stream entrance (YS 1) and the trough (YS3), compared to other survey sites. There was a discrepancy in the number of species collected during April and October, but the overall species count remained consistent. Moreover, the autumn season exhibited a higher abundance of fish, with 381 individuals observed compared to 285 in the spring. Amongst the species observed, the number of *Hemiculter leucisculus* individuals increased from 72 in the spring to 148 in autumn.

Comparing the detectability Eurasian Otters using eDNA: Metabarcoding vs. qPCR

The mammal list derived from metabarcoding results showed a total of six families and 10 species (Table 3). Negative samples, which were amplified to under 50,000 reads after MiSeq sequencing, indicated that there was no

Table 2. The fish species list identified from field survey in the study area (four families and 16 species).

Scientific name	Conventional survey in Apr								Conventional survey in Oct							
	YS1	YS2	YS3	YS4	YS5	YS6	YS7	RA(%)	YS1	YS2	YS3	YS4	YS5	YS6	YS7	RA(%)
Cyprinidae																
Abbottina rivularis	2	1						1.05	6	2						2.1
Acheilognathus macropterus	9	2			2	6	5	8.42	2	5			3	8	6	6.3
Carassius auratus	3	1	2	9	7	2	1	8.77	5	2	12	3	6	1	3	8.4
Cyprinus carpio	2	4	27	11	4	3	3	18.95	1	2	35	9	2	5	1	14.44
Erythroculter erythropterus	5	3		2	1	1	2	4.91	2	1		3		1	5	3.15
Hemibarbus labeo	1							0.35	4							1.05
Hemiculter leucisculus	16	7	15	12	8	3	11	25.26	56	21	10	26	12	5	18	38.85
Pseudorasbora parva	4	3	2	3	1		1	4.91	3	1	1	1	2		4	3.15
Squalidus japonicus coreanns	7	2		3	2			4.91	9	2		2	1			3.67
Zacco platypus			12					4.21	2		19					5.51
Centrarchidae																
Lepomis macrochirus			6	2				2.81			3	8				2.89
Micropterus salmoides			3					1.05			9					2.36
Gobiidae																
Gymnogobius urotaenia	9	2	6	2	1			7.02	5	3	3	1	2			3.67
Rhinogobius giurinus	3		4			2		3.16	1	1	2			3		1.84
Tridentiger brebispinis	2	1	8					3.86	3	1	5					2.36
Siluridae																
Silurus asotus	1							0.35			1					0.26
Total number of individuals	64	26	85	44	26	17	23		99	41	100	53	28	23	37	
Total number of species	14	11	11	9	9	7	7		14	12	12	9	8	7	7	

cross-contamination between samples. The eDNA survey revealed 6 of 13 species recorded in fieldworks and literature reviews, but there was no detection of Eurasian otters in both eDNA results (Fig. 2). Throughout surveys in April and October, *Sus scrofa* accounted for the highest percentage of reads (88.2%, 548,880 reads), followed by *Bos taurus* (6.7%, 41,745 reads), *Ovis aries* (2.9%, 18,078 reads) and *Mus musculus* (1.4%, 8,541 reads). It is estimated that genetic materials from domestic animals commonly consumed by people in the city were detected by the eDNA survey. In addition, the common urban species like the Muridae family were consistently identified by metabarcoding across all sampling sites and seasons.

In contrast, the qPCR assay amplified Eurasian otters from the same samples analysed by metabarcoding. The LoD of the assay confirmed by the standard curve (Slope = -3.108, Y-intercept = 24.623, R^2 = 0.949, Eff% = 109.773), was determined to be 0.1 ng/µl DNA (Suppl. material 2). Positive samples were evaluated by the LoD calculated from the standard curve. Of the 10 PCR repetitions with 21 samples, five samples were positive for all 10 times, while nine samples were all negative. The trend line between the detection rate and Ct value showed was inversely proportional (Slope = -2.843 Y-intercept = 29.154, R^2 = 0.74) (Fig. 3), suggesting that the number of qPCR repetitions could be determined according to the concentration of samples and their detection rate. For instance, to achieve 100% of detection, the concentration of

eDNA sample should exceed a cycle threshold of 26.3. With a high confidence level and considering the criteria, Eurasian otters were detected at three of seven sites (42.9%) in April (Fig. 4a). When thresholds were not considered, the number of detections increased to six of seven sites (85.7%). The repetition threshold with a high repetition number is more influential than the sample threshold in determining the number of detections. However, in October, despite differences in detection times amongst sample repetitions, eDNA detection confirmed Eurasian otters at all sampling sites (100.0%), regardless of the confidence level (Fig. 4b).

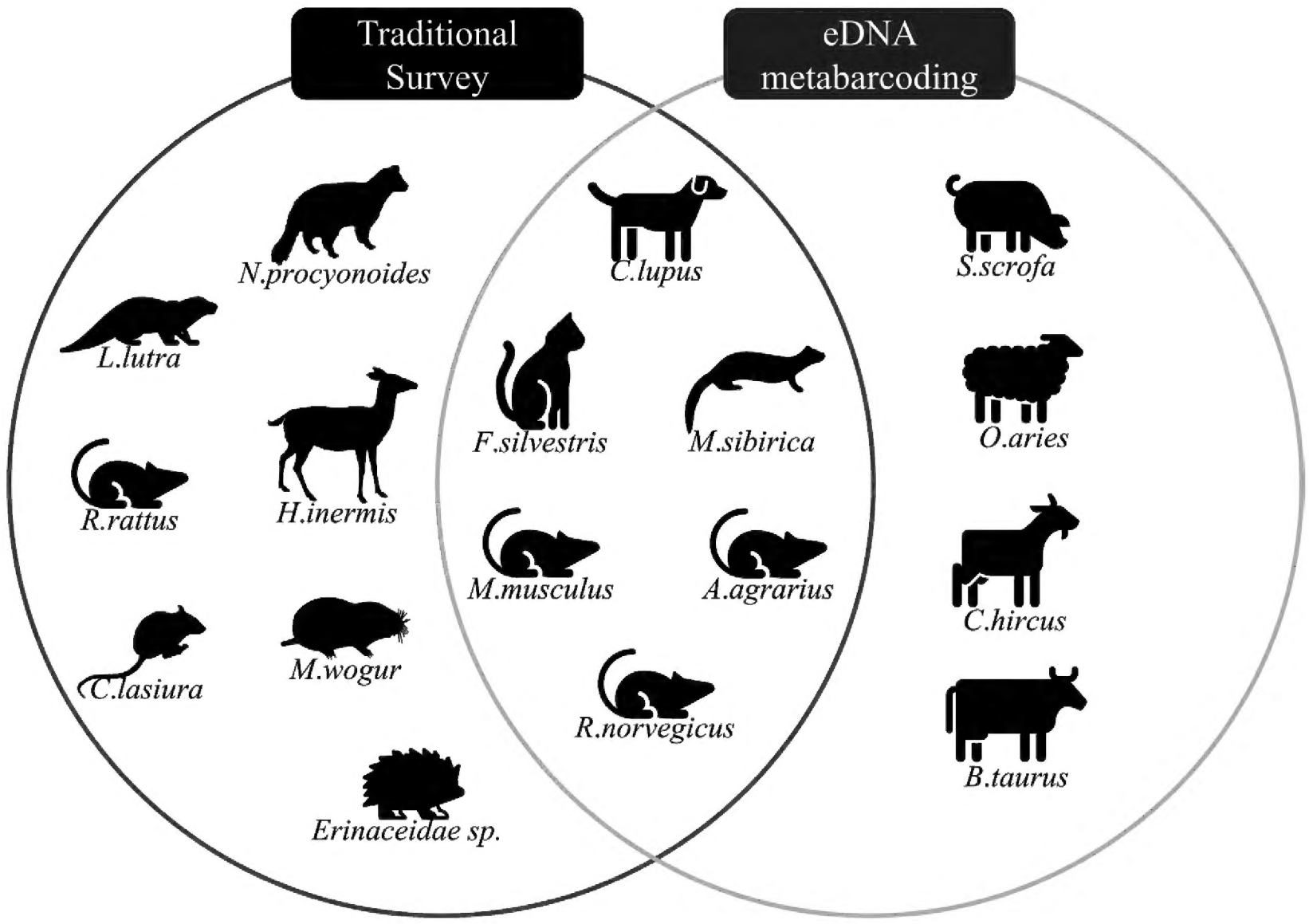

Figure 2. The comparison of identified mammal species between traditional survey and eDNA metabarcoding.

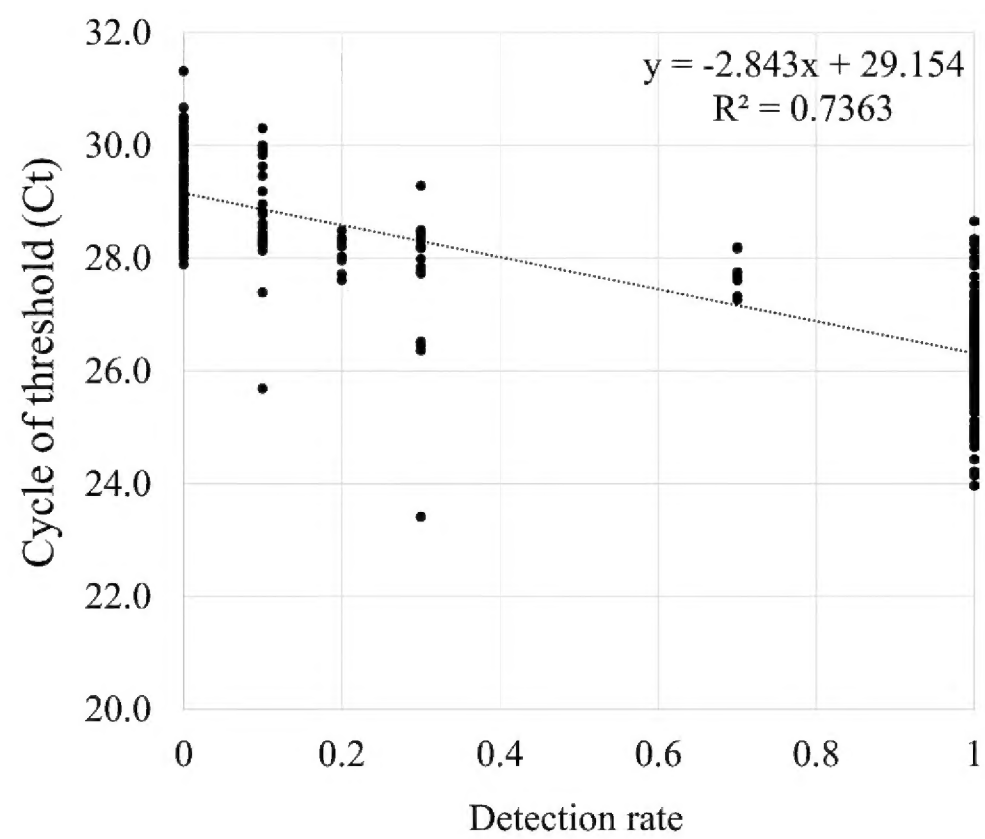

Figure 3. The regression plot between the detection rate and a cycle of threshold (Ct).

Table 3. The detected mammal list by eDNA metabarcoding in April and October.

Scientific name	eDNA survey in Apr							eDNA survey in Oct						
	YS1	YS 2	YS3	YS4	YS5	YS6	YS7	YS1	YS2	YS3	YS4	YS5	YS6	YS7
Bovidae														
Bos taurus				6527	1722	9738		3079	736	6330	804	10405	2404	
Ovis aries	1483	7065		7214		806	478	298		734				
Capra hircus				423										
Canidae														
*Canis lupus familiaris**	11			51	19	71		293	226	230	20	184	138	
Felidae														
*Felis silvestris**								162	11			8	68	
Muridae														
*Apodemus agrarius **								58						
*Mus musculus**	1982	753	2862	244	375	575	1359				341	50		
*Rattus norvegicus**	44				1234	321	77	98	170	108	602	478	59	
Mustelidae											10			
*Mustela sibirica**											10			
Suidae														
Sus scrofa	9068	52483	1169	250899	28584	129353	3483	27302	5739	21900	13181	3451	1178	1090
The number of species	5	3	2	6	5	6	4	7	5	5	7	6	5	1

*, The recorded species in fieldwork and literature reviews.

The evaluation of distribution pattern and food species estimation

Distribution patterns of *L. lutra* by seasons

As the detection probability of Eurasian otters is high in October, it is estimated that Eurasian otters expanded their home range during this month compared to April (Fig. 5). In April, without applying thresholds, otters were detected at six sites, except for the YS4 site located in the middle of Saetgang. The average detection ratio at these sites was 37.8 ± 30.2% (mean ± SD). When thresholds were applied to determine positive samples, YS6 and YS7 were classified as absent sites, while the average detection ratio for positive samples increased to 95.0 ± 11.2% (mean ± SD). In October, all sites indicated the presence of Eurasian otters, showing a high average detection ratio of 91.0 ± 14.3% (mean ± SD) without a threshold and 100.0% with a threshold.

The estimation of potential food species for *L. lutra* in Saetgang

As Eurasian otters are known to prefer fish, making up over 70% of their diet (Suppl. material 5), only fish species identified in the eDNA metabarcoding results were considered potential food species in this study (Krawczyk et al. 2016; Narváez et al. 2020). A total of 3,898,508 and 1,089,451 reads, identified as fish species, were recorded in April and October, respectively. After excluding alien species not recorded in Korea and those unsuitable for freshwater, we retained

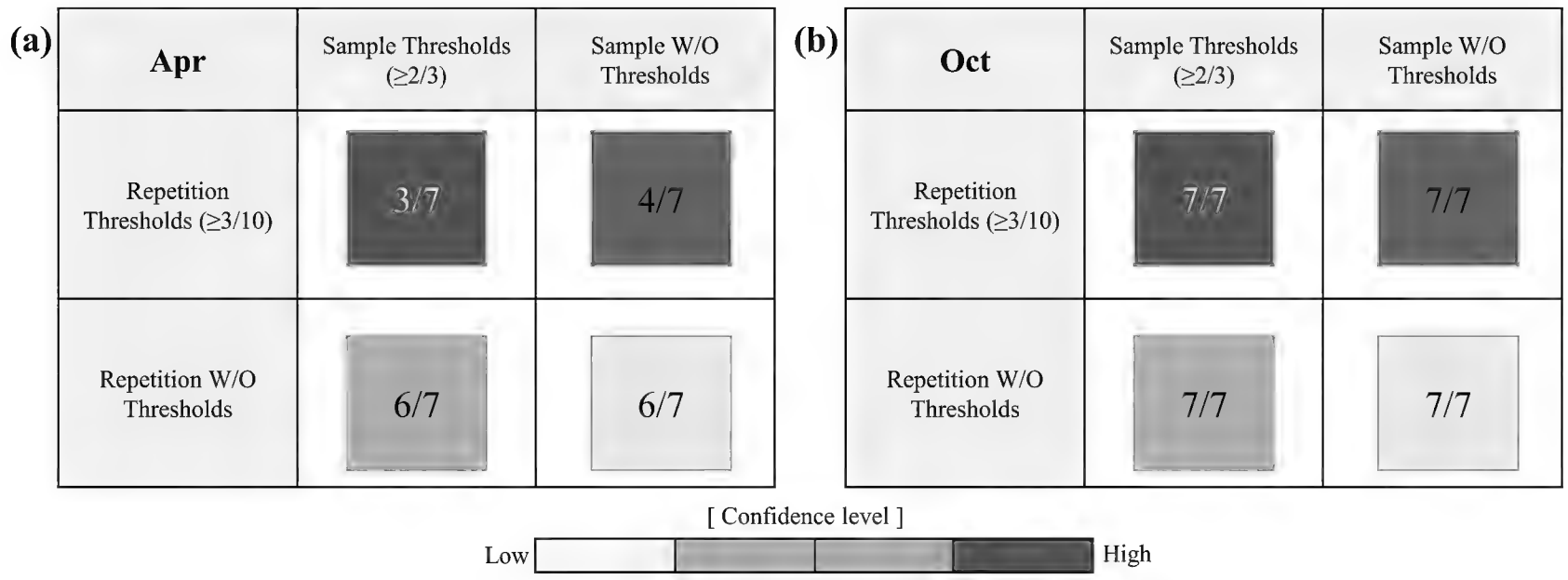

Figure 4. The number of detections according to criterion in April (**a**) and October (**b**). The reliability level was determined, based on the criterion that reliability increases when the threshold value is applied.

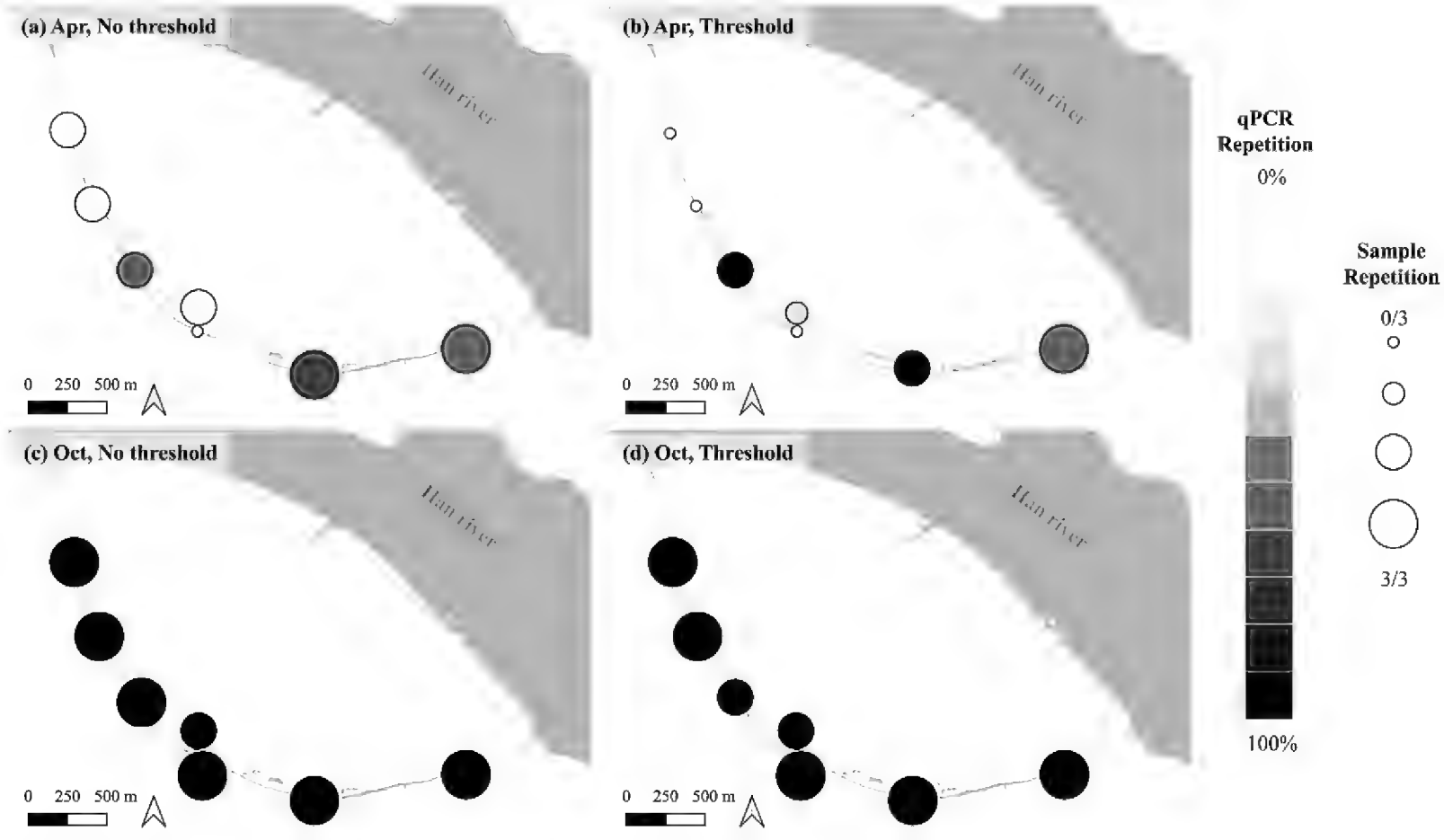

Figure 5. The distribution pattern of Eurasian otters according to seasons, based on eDNA detection with qPCR.

3,039,297 (Apr) and 748,001 (Oct) reads, constituting 78.0% and 68.7% of the raw data, respectively (Suppl. material 6). A total of 15 families and 52 species were detected. In both seasons, Cyprinidae accounted for the largest proportion of fish species at the family level (Apr, 54.57%; Oct, 43.58%), followed by Gobiidae (Apr, 16.90%; Oct, 22.88%) (Fig. 6). At the species level, *Pseudorasbora parva* was the dominant species in Saetgang, representing 5.68% and 6.35% of the natural log-transformed total number of reads (Suppl. material 7). Additionally, *Tridentiger obscurus* (5.16%), *Acheilognathus macropterus* (5.16%), *Acheilognathus chankaensis* (4.65%) and *Cyprinus carpio* (4.22%) were prominent in April. In October, *A.chankaensis* (5.05%) and *T. obscurus* (4.97%) were prevalent and *Hemibarbus sp.* (4.82%) and *Zacco platypus* (4.39%) also appeared. Similarly, in the conventional collection-based survey, Cyprinidae (Apr, 81.75%, Oct, 86.61%) and Gobiidae (Apr, 14.04%, Oct, 7.87%) were the dominant families (Table 2). At the species level, there was little difference in species with high relative abundance including *H. leucisculus*, but it displayed proportions similar to several other species, such as *A. macropterus*, *C. carpio*, *P. parva* and *Z. platypus*.

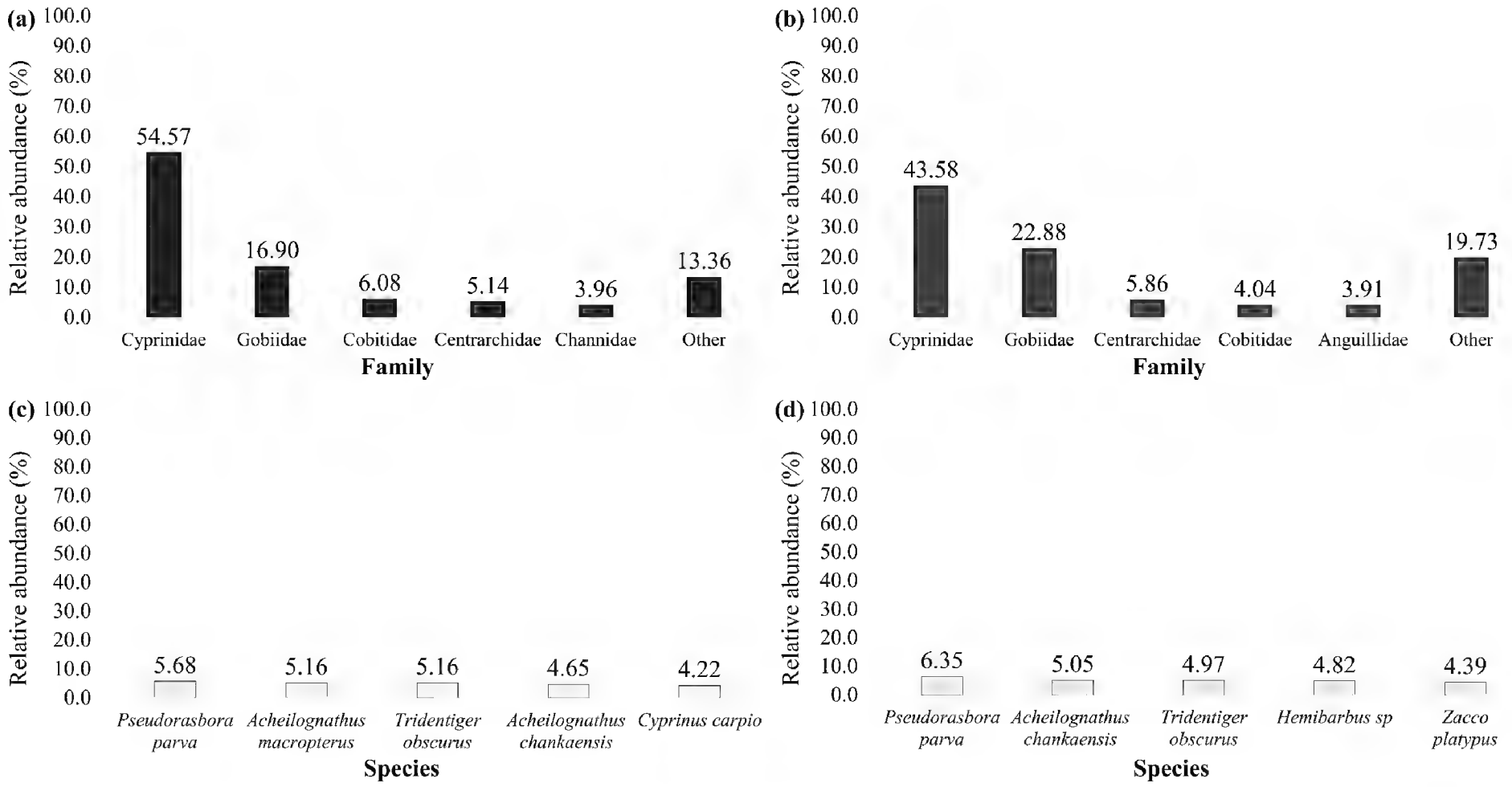

Figure 6. The relative abundance (%) of detected fish species at the family level and species level in April and October **a** family level in April **b** family level in October **c** species level in April **d** species level in October.

Discussion

The ecological characteristics of Eurasian otters in the Yeouido Ecological Park

Trace tracking survey and qPCR results confirmed the presence of Eurasian otters in Yeouido Ecological Park. Furthermore, the increased detection probability and the discovery of footprints in October suggest that Eurasian otters expand their home range during autumn. Eurasian otters are known to breed at times of the year when conditions are favourable, typically in early spring between February and March in Korea (Heggberget and Christensen 1994; National Institute of Biological Resources 2018). Since gestation lasts 60 to 70 days and weaning occurs at 3 months, it is estimated that the home range was reduced in April for nurturing purposes, coinciding with a decrease in detection probability (Han and Han 2022). Moreover, the concentration of DNA has been used in several studies to estimate biomass or population sizes (Takahara et al. 2012; Di Muri et al. 2020; Rourke et al. 2022). Therefore, the distribution patterns of Eurasian otters can be determined by the eDNA concentration further from the appearance location (Figs 5, 7). The eDNA survey conducted in October revealed that *L. lutra* was identified at all study sites. The eDNA concentration indicated that the density of *L. lutra* was higher at sites YS1 and YS2 compared to other sites, especially the lentic site YS3.

As opportunistic predators, otters are likely to exploit locally abundant food sources, adapting their diet to seasonal variations and differences in habitat type (Ruiz-Olmo et al. 2001; Kloskowski et al. 2013). An analysis of prey preferences in Eurasian otters, based on spraint analysis from freshwater and estuarine environments in Korea, revealed that families Cyprinidae, Channidae and Centrarchidae exhibited the highest detection rates (Lee 2012; Kumari et al. 2019). In contrast, species from the orders Mugiliformes, Gadiformes and

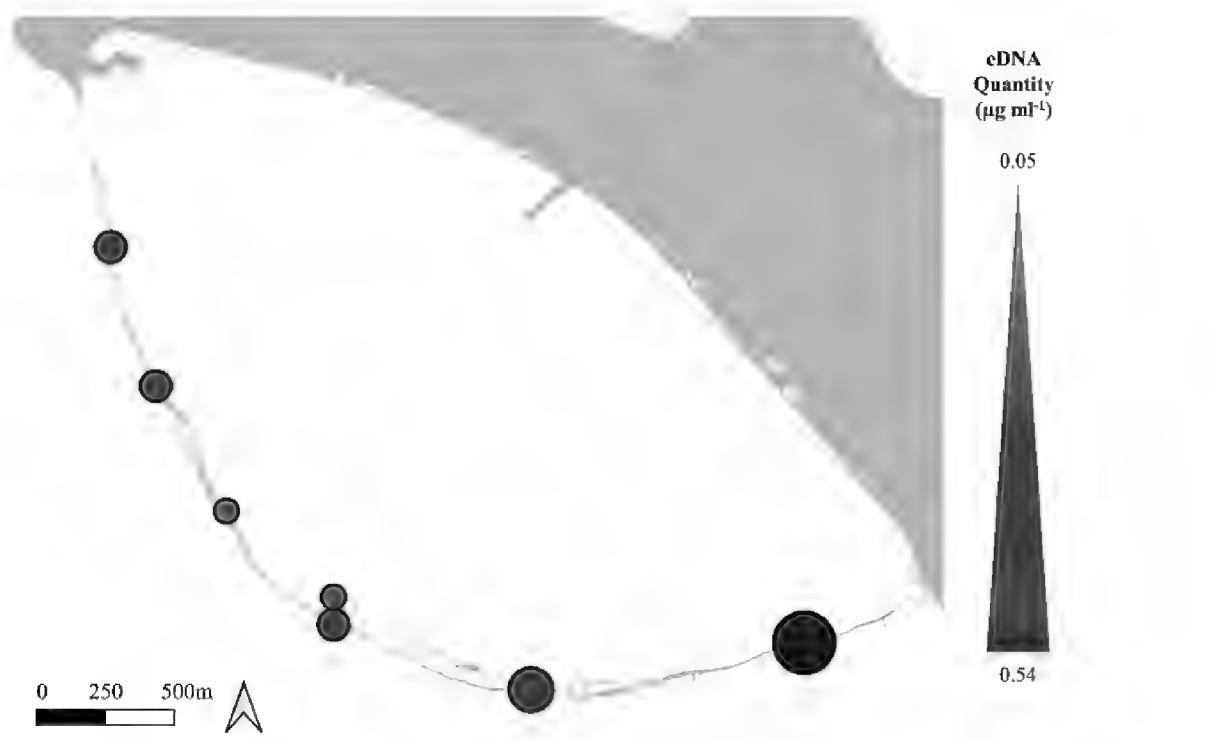

Figure 7. Estimates of *L.lutra* density at the sampling site based on eDNA concentration in October.

Pleuronectiformes were more prevalent in marine environments (Choi and Yoon 2012). Notably, the dominance of Cyprinidae, particularly *P. parva*, in April and October at this study area suggests a high availability of these species as a food source for Eurasian otters. Although environmental DNA surveys offer valuable insights into the habitat and ecological characteristics of species, it is essential to acknowledge and address potential biases that may influence these surveys.

How to improve the detection probability and reliability of rare species

In addition to implementing repeated site sampling and qPCR analysis in the lab to enhance the stability and reliability of PCR results, it is crucial to address challenges, such as the presence of inhibitors and primer efficiency in eDNA surveys (Macher et al. 2021). eDNA surveys enable highly sensitive detection of species that are low in abundance. However, the sensitivity of eDNA detection, especially with low sample concentrations, introduces uncertainties in presence-absence evaluations (Harper et al. 2018; Yu et al. 2022).

This study conducted three independent samplings at each site and ten independent qPCR analyses per sample. Findings showed that, in the 10 repetitions, 71.4% of the samples detected less than three times were considered positive, while those detected more than seven times accounted for the remaining 28.6%. This indicates the necessity of a minimum of three repeats to enhance detection probability. The decision-making process for establishing presence-absence criteria in qPCR assays can, therefore, be tailored, based on these results, with a high threshold for confident presence data or a lower threshold when determining broader habitat occupancy.

Although this study achieved high consistency in qPCR results without proceeding with inhibitor removal, eliminating or reducing inhibitors is vital for achieving high-quality detection results. Field methods, such as collecting small sampling volumes, using large pore-sized filters and increasing sampling intensity, help mitigate inhibitors (U.S. Fish and Wildlife Service 2022). However, these methods might reduce the concentration of target eDNA, necessitating careful application (Harper et al. 2019b). In the lab, commercial master mixes

that include inhibitor removal columns and PCR enhancers provide additional tools to counteract inhibitors (Hunter et al. 2019; Sanches and Schreier 2020).

Additionally, understanding the characteristics of the study area, such as Yeouido Saetgang Ecological Park — an urban park vulnerable to genetic pollution from wastewater and artificial factors — is crucial before deciding on the analysis strategy (Lee et al. 2021). This study also found that otters were not detected and industrial and companion animals were frequently identified through eDNA metabarcoding. The study's limitations due to cost and sample amount prevented repetition in metabarcoding, thus restricting comparisons between metabarcoding and qPCR detection rates. Moreover, the efficiency of the MiMammal universal primer was not evaluated against the Lutcyt species-specific primer for Eurasian otters. Although prior research has demonstrated that MiMammal primers can detect Eurasian otters (Ushio et al. 2017; Sales et al. 2020; Kim et al. 2021), further assessments focusing on comparative performance, environmental and sample type suitability and quantitative capabilities are required to improve result robustness (Bustin and Huggett 2017; Kumar et al. 2022).

Limitations and potentials for habitat evaluation and distribution patterns of semi-aquatic mammals with eDNA survey

Compared to other terrestrial mammals, semi-aquatic mammals spend most of their time in the aquatic environment engaging in activities, such as foraging and reproduction. This makes it easier to analyse environmental DNA (eDNA) through water samples. In other words, estimating the distribution and density of these species can be straightforward when obtaining eDNA samples with a certain concentration or higher. However, it is essential to consider the characteristics of the eDNA survey, including degradation, suspension and sedimentation, which can affect eDNA detection and concentration. eDNA particles share similar transport dynamics with fine particulate organic matter (Harrison et al. 2019). Lentic systems, such as lakes and ponds, are primarily influenced by gravitational settling and can disperse eDNA about 100 m (Nevers et al. 2020). Additionally, eDNA concentration in aquatic environments is commonly influenced or degraded by physiochemical factors, such as water turbidity, stratification caused by water temperature and benthic substrate (Deiner and Altermatt 2014; Shogren et al. 2017). Biological factors also play a crucial role. The production of eDNA varies not only with the biomass of the target species or community size, but also with species interactions like predation or competition (Stewart 2019). Therefore, the consideration of abiotic and biotic factors affecting eDNA detection should be reflected in the eDNA sampling and analysis strategy. Moreover, since the most reliable evaluation method involves assessing presence-absence through trace investigation or camera trapping and identifying the source of food through analysis of spraints and stomach contents, it is necessary to complement the eDNA investigation with these traditional methods.

Conclusions

Our study aimed to compare environmental DNA (eDNA) analysis methods for detecting Eurasian otters in urban areas. We utilised both eDNA metabarcoding and qPCR techniques to gain insights into the ecological

characteristics of Eurasian otters, including their seasonal distribution patterns and potential prey availability. Our research highlights the effectiveness of qPCR in elucidating otter distribution patterns in urban environments and the utility of metabarcoding in characterising the biological environment. In contrast to metabarcoding, which failed to detect otters, qPCR successfully identified otters at a minimum of three sites within the study area, demonstrating its superior efficacy in assessing otter distribution. qPCR analysis revealed that otters expanded their home range more significantly in October than in April. Additionally, metabarcoding analysis indicated that Cyprinidae, particularly *Pseudorasbora parva*, exhibited the highest prey availability. However, our study also underscores the importance of meticulously selecting eDNA analysis techniques, especially in environments dominated by certain species. We discovered that pigs (*Sus scrofa*) comprised over 88.2% of the mammals detected through metabarcoding, highlighting the risk of species-masking effects. This emphasises the need for employing customised eDNA analysis methods suited to the characteristics of the study area. Our study contributes not only to understanding the ecological characteristics of Eurasian otters, but also to enhancing the accuracy and reproducibility of eDNA surveys, thereby enriching our understanding of ecological dynamics in aquatic ecosystems.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: YK. Funding acquisition: YS. Investigation: YK. Supervision: YS. Writing - original draft: YK. Writing - review and editing: YS, SH.

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Data availability

All the data supporting the findings of this study are available in the Suppl. material 6. Raw sequence data from the metabarcoding dataset are available in the Sequence Read Archive (SRA) under the accession number PRJNA1127811.

References

Bohmann K, Evans A, Gilbert MTP, Carvalho GR, Creer S, Knapp M, Yu DW, de Bruyn M (2014) Environmental DNA for wildlife biology and biodiversity monitoring. Trends in Ecology & Evolution 29(6): 358–367. https://doi.org/10.1016/j.tree.2014.04.003

Breton BAA, Beaty L, Bennett AM, Kyle CJ, Lesbarrères D, Vilaça ST, Wikston MJH, Wilson CC, Murray DL (2022) Testing the effectiveness of environmental DNA (eDNA) to quantify larval amphibian abundance. Environmental DNA 00(6): 1–12. https://doi.org/10.1002/edn3.332

Bruce K, Blackman R, Bourlat SJ, Hellström AM, Bakker J, Bista I, Bohmann K, Bouchez A, Brys R, Clark K, Elbrecht V, Fazi S, Fonseca V, Hänfling B, Leese F, Mächler E, Mahon AR, Meissner K, Panksep K, Pawlowski J, Schmidt Yáñez P, Seymour M, Thalinger B, Valentini A, Woodcock P, Traugott M, Vasselon V, Deiner K (2021) A practical guide to DNA-based methods for biodiversity assessment. A practical guide to DNA-based methods for biodiversity assessment, 94 pp. https://doi.org/10.3897/ab.e68634

Bustin S, Huggett J (2017) qPCR primer design revisited. Biomolecular Detection and Quantification 14: 19–28. https://doi.org/10.1016/j.bdq.2017.11.001

Buxton AS, Groombridge JJ, Griffiths RA (2018) Seasonal variation in environmental DNA detection in sediment and water samples. PLoS ONE 13(1): 1–14. https://doi.org/10.1371/journal.pone.0191737

Choi J-W, Yoon M-H (2012) A Study on Food Habits of the Otter, Lutra lutra, and Effects of Construction of the Busan New Port on its Prey. Journal of Life Science 22(6): 736–743. https://doi.org/10.5352/JLS.2012.22.6.736

Da Silva Neto JG, Sutton WB, Spear SF, Freake MJ, Kéry M, Schmidt BR (2020) Integrating species distribution and occupancy modeling to study hellbender (Cryptobranchus alleganiensis) occurrence based on eDNA surveys. Biological Conservation 251: 108787. https://doi.org/10.1016/j.biocon.2020.108787

Davy CM, Kidd AG, Wilson CC (2015) Development and validation of environmental DNA (eDNA) markers for detection of freshwater turtles. PLoS ONE 10(7): 1–13. https://doi.org/10.1371/journal.pone.0130965

Deiner K, Altermatt F (2014) Transport Distance of Invertebrate Environmental DNA in a Natural River. PLoS ONE 9(2): e88786. https://doi.org/10.1371/journal.pone.0088786

Di Muri C, Handley LL, Bean CW, Li J, Peirson G, Sellers GS, Walsh K, Watson HV, Winfield IJ, Hänfling B (2020) Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. Metabarcoding and Metagenomics 4: 97–112. https://doi.org/10.3897/mbmg.4.56959

Dickie IA, Boyer S, Buckley HL, Duncan RP, Gardner PP, Hogg ID, Holdaway RJ, Lear G, Makiola A, Morales SE, Powell JR, Weaver L (2018) Towards robust and repeatable sampling methods in eDNA-based studies. Molecular Ecology Resources 18(5): 940–952. https://doi.org/10.1111/1755-0998.12907

Dou H, Wang M, Yin X, Feng L, Yang H (2023) Can the Eurasian otter (Lutra lutra) be used as an effective sampler of fish diversity? Using molecular assessment of otter diet to survey fish communities. Metabarcoding and Metagenomics 7: 19–35. https://doi.org/10.3897/mbmg.7.96733

Edgar RC, Bateman A (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19): 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Erlinge S (1967) Home Range of the Otter Lutra lutra L. in Southern Sweden. Oikos 18(2): 186. https://doi.org/10.2307/3565098

Forstchen M (2020) The Elephant in the Lab (and Field): Contamination in Aquatic Environmental DNA Studies. Frontiers in Ecology and Evolution 8: 609973. https://doi.org/10.3389/fevo.2020.609973

Gang HS, I BG, Han DH, Gim HR, Jo SC, Gim TH (2015) A Study on the Management and Utilization of Saetgang River (I). KEI(Hanguk Hwangyeong Jeongchaek·Pyeongga Yeonguwon), Sejong.

Han SW, Han SY (2022) Current Status of Korean Otter and Their Conservation., Vol.3, No.1, 1–6. https://doi.org/10.22920/PNIE.2022.3.1.1

Harper LR, Lawson Handley L, Hahn C, Boonham N, Rees HC, Gough KC, Lewis E, Adams IP, Brotherton P, Phillips S, Hänfling B (2018) Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (Triturus cristatus). Ecology and Evolution 8(12): 6330–6341. https://doi.org/10.1002/ece3.4013

Harper LR, Lawson Handley L, Carpenter AI, Ghazali M, Di Muri C, Macgregor CJ, Logan TW, Law A, Breithaupt T, Read DS, McDevitt AD, Hänfling B (2019a) Environmental DNA (eDNA) metabarcoding of pond water as a tool to survey conservation and management priority mammals. Biological Conservation 238: 108225. https://doi.org/10.1016/j.biocon.2019.108225

Harper LR, Buxton AS, Rees HC, Bruce K, Brys R, Halfmaerten D, Read DS, Watson HV, Sayer CD, Jones EP, Priestley V, Mächler E, Múrria C, Garcés-Pastor S, Medupin C, Burgess K, Benson G, Boonham N, Griffiths RA, Lawson Handley L, Hänfling B (2019b) Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. Hydrobiologia 826(1): 25–41. https://doi.org/10.1007/s10750-018-3750-5

Harper LR, Watson H V., Donnelly R, Hampshire R, Sayer CD, Breithaupt T, Hänfling B (2020) Using DNA metabarcoding to investigate diet and niche partitioning in the native European otter (Lutra lutra) and invasive American mink (Neovison vison). Metabarcoding and Metagenomics 4: e56087. https://doi.org/10.3897/mbmg.4.56087

Harrison JB, Sunday JM, Rogers SM (2019) 286 Predicting the fate of eDNA in the environment and implications for studying biodiversity. Royal Society Publishing, 1–9. https://doi.org/10.1098/rspb.2019.1409

Heggberget TM, Christensen H (1994) Reproductive Timing in Eurasian Otters on the Coast of Norway 17: 339–348. https://doi.org/10.1111/j.1600-0587.1994.tb00111.x [March 21, 2023]

Hernandez C, Bougas B, Perreault-Payette A, Simard A, Côté G, Bernatchez L (2020) 60 specific eDNA qPCR assays to detect invasive, threatened, and exploited freshwater vertebrates and invertebrates in Eastern Canada. Environmental DNA 2(3): 373–386. https://doi.org/10.1002/edn3.89

Hunter ME, Ferrante JA, Meigs-Friend G, Ulmer A (2019) Improving eDNA yield and inhibitor reduction through increased water volumes and multi-filter isolation techniques. Scientific Reports 2019 9: 1–9. https://doi.org/10.1038/s41598-019-40977-w

Ishige T, Miya M, Ushio M, Sado T, Ushioda M, Maebashi K, Yonechi R, Lagan P, Matsubayashi H (2017) Tropical-forest mammals as detected by environmental DNA at natural saltlicks in Borneo. Biological Conservation 210: 281–285. https://doi.org/10.1016/j.biocon.2017.04.023

Jamwal PS, Bruno A, Galimberti A, Magnani D, Casiraghi M, Loy A (2023) Environmental DNA revealed high accuracy in detection of the Eurasian otter in Himalaya. Aquatic Conservation 33(11): 1309–1320. https://doi.org/10.1002/aqc.4010

Jang-Liaw NH (2021) A barcoding-based scat-analysis assessment of Eurasian otter Lutra lutra diet on Kinmen Island. Ecology and Evolution 11(13): 8795–8813. https://doi.org/10.1002/ece3.7712

Jo YS, Won C (2019) The Sprainting Behavior and Habitat Preference of the Eurasian Otter Lutra lutra Along a Montane Stream in South Korea. Mammal Study 45(1): 3–11. https://doi.org/10.3106/ms2018-0071

Kim IS (1997) Ministry of Education Illustrated encyclopedia of fauna and flora of Korea. Vol.37: Freshwater fishes. Chungcheongnam-do, 629 pp. https://library.krihs.re.kr/search/detail/CATTOT000000066605 [June 21, 2021]

Kim J (2018) Seasonal differences in diet and activity of the Eurasian Otter (Lutra lutra). Seoul National University.

Kim I-S, Park JY (2002) Freshwater Fish of Korea. Kyohak Publishing Co., Seoul, Korea, 466 pp.

Kim IS, Choi Y, Lee CL, Lee YJ, Kim BJ, Kim JH (2005) Illustrated Book of Korean Fishes. Kyohak Publishing Co., Seoul, Korea, 615 pp.

Kim Y, Han Y, Park J, Kim H, Cho S, Song Y (2021) Monitoring the presence of wild boar and land mammals using environmental DNA metabarcoding - Case study in Yangpyeong-gun, Gyeonggi-do -. Journal of the Korean Society of Environmental Restoration Technology 24: 133–144. https://doi.org/10.13087/KOSERT.2021.24.6.133

Kloskowski J, Rechulicz J, Jarzynowa B (2013) Resource availability and use by Eurasian otters Lutra lutra in a heavily modified river-canal system. Wildlife Biology 19(4): 439–451. https://doi.org/10.2981/12-104

Krawczyk AJ, Bogdziewicz M, Majkowska K, Glazaczow A (2016) Diet composition of the Eurasian otter Lutra lutra in different freshwater habitats of temperate Europe: A review and meta-analysis. Mammal Review 46(2): 106–113. https://doi.org/10.1111/mam.12054

Krehenwinkel H, Wolf M, Lim JY, Rominger AJ, Simison WB, Gillespie RG (2017) Estimating and mitigating amplification bias in qualitative and quantitative arthropod metabarcoding. Scientific Reports 7: 1–12. https://doi.org/10.1038/s41598-017-17333-x

Kumar G, Reaume AM, Farrell E, Gaither MR (2022) Comparing eDNA metabarcoding primers for assessing fish communities in a biodiverse estuary. PLoS ONE 17(6): e0266720. https://doi.org/10.1371/journal.pone.0266720

Kumari P, Dong K, Eo KY, Lee WS, Kimura J, Yamamoto N (2019) DNA metabarcoding-based diet survey for the Eurasian otter (Lutra lutra): Development of a Eurasian otter-specific blocking oligonucleotide for 12S rRNA gene sequencing for vertebrates. PLoS ONE 14(12): e0226253. https://doi.org/10.1371/journal.pone.0226253

Lee S (2012) Studies on Food Items of River Otter Residing in the Hongchon Stream. Journal of Wetlands Researh 14: 591–596. https://www.earticle.net/Article/A198921 [March 21, 2023]

Lee GJ, Cho HJ, Na JE, Jang JM, Jang JW, Lee HY (2020) Stress Responses of Eurasian Otter (Lutra lutra) in Relation to Habitats and Season. Korean Journal of Ecology and Environment 53(1): 80–90. https://doi.org/10.11614/KSL.2020.53.1.080

Lee HT, Liao CH, Hsu TH (2021) Environmental DNA (eDNA) metabarcoding in the fish market and nearby seafood restaurants in taiwan reveals the underestimation of fish species diversity in seafood. Biology (Basel) 10(11): 1132. https://doi.org/10.3390/biology10111132

Lintermans M (2016) Finding the needle in the haystack: Comparing sampling methods for detecting an endangered freshwater fish. Marine and Freshwater Research 67(11): 1740–1749. https://doi.org/10.1071/MF14346

Loy A, Kranz A, Oleynikov A, Roos A, Savage M&, Duplaix N (2022) *Lutra lutra* (amended version of 2021 assessment). https://doi.org/10.2305/IUCN.UK.2022-2.RLTS.T12419A218069689.en

Macher TH, Schütz R, Arle J, Beermann AJ, Koschorreck J, Leese F (2021) Beyond fish eDNA metabarcoding: Field replicates disproportionately improve the detection of

stream associated vertebrate species. Metabarcoding and Metagenomics 5: e66557. https://doi.org/10.3897/mbmg.5.66557

Mariani S, Harper LR, Collins RA, Baillie C, Wangensteen OS, McDevitt AD, Heddell-Cowie M, Genner MJ (2021) Estuarine molecular bycatch as a landscape-wide biomonitoring tool. Biological Conservation 261: 109287. https://doi.org/10.1016/j.biocon.2021.109287

Mauvisseau Q, Burian A, Gibson C, Brys R, Ramsey A, Sweet M (2019) Influence of accuracy, repeatability and detection probability in the reliability of species-specific eDNA based approaches. Scientific Reports 9(1): 580. https://doi.org/10.1038/s41598-018-37001-y

Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, Minamoto T, Yamamoto S, Yamanaka H, Araki H, Kondoh M, Iwasaki W (2015) MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. Royal Society Open Science 2(7): 150088. https://doi.org/10.1098/rsos.150088

Miya M, Gotoh RO, Sado T (2020) MiFish metabarcoding: a high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. Fisheries Science 86: 939–970. https://doi.org/10.1007/s12562-020-01461-x

Muha TP, Rodríguez-Rey M, Rolla M, Tricarico E (2017) Using environmental DNA to improve species distribution models for freshwater invaders. Frontiers in Ecology and Evolution 5: 158. https://doi.org/10.3389/fevo.2017.00158

Narváez M, Cabezas S, Blanco-Garrido F, Baos R, Clavero M, Delibes M (2020) Eurasian otter (Lutra lutra) diet as an early indicator of recovery in defaunated river communities. Ecological Indicators 117: 106547. https://doi.org/10.1016/j.ecolind.2020.106547

National Institute of Biological Resources (2018) Endangered species at a glance. Hwangyeongbu Gungnip Saengmul Jawongwan.

National Institute of Biological Resources (2021) Red Data Book of Republic of Korea (second edition) Volume 4. Mammals. Incheon

National Institute of Environmental Research (2016) Biomonitoring Survey and Assessment Manual. http://www.nier.go.kr

Néill LÓ, Veldhuizen T, de Jongh A, Rochford J (2009) Ranging behaviour and socio-biology of Eurasian otters (Lutra lutra) on lowland mesotrophic river systems. European Journal of Wildlife Research 55(4): 363–370. https://doi.org/10.1007/s10344-009-0252-9

Nevers MB, Przybyla-Kelly K, Shively D, Morris CC, Dickey J, Byappanahalli MN (2020) Influence of sediment and stream transport on detecting a source of environmental DNA. PLoS ONE 15(12): e0244086. https://doi.org/10.1371/journal.pone.0244086

Park HC, Han TY, Kim DC, Min MS, Han SY, Kim KS, Lee H (2011) Individual identification and sex determination of Eurasian otters (Lutra lutra) in Daegu city based on genetic analysis of otter spraint. Genes & Genomics 33(6): 653–657. https://doi.org/10.1007/s13258-011-0051-z

Raymaekers M, Smets R, Maes B, Cartuyvels R (2009) Checklist for optimization and validation of real-time PCR assays. Journal of Clinical Laboratory Analysis 23(3): 145–151. https://doi.org/10.1002/jcla.20307

Riaz M, Kuemmerlen M, Wittwer C, Cocchiararo B, Khaliq I, Pfenninger M, Nowak C (2020) Combining environmental DNA and species distribution modeling to evaluate reintroduction success of a freshwater fish. Ecological Applications 30(2): e02034. https://doi.org/10.1002/eap.2034

Ritter CD, Dal Pont G, Stika PV, Horodesky A, Cozer N, Netto OSM, Henn C, Ostrensky A, Pie MR (2022) Wanted not, wasted not: Searching for non-target taxa in environmental DNA metabarcoding by-catch. Environmental Advances 7: 100169. https://doi.org/10.1016/j.envadv.2022.100169

Rourke ML, Fowler AM, Hughes JM, Broadhurst MK, DiBattista JD, Fielder S, Wilkes Walburn J, Furlan EM (2022) Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. Environmental DNA 4(1): 9–33. https://doi.org/10.1002/edn3.185

Ruiz-Olmo J, López-Martín JM, Palazón S (2001) The influence of fish abundance on the otter (Lutra lutra) populations in Iberian Mediterranean habitats. Journal of Zoology (London, England) 254(3): 325–336. https://doi.org/10.1017/S0952836901000838

Sales NG, McKenzie MB, Drake J, Harper LR, Browett SS, Coscia I, Wangensteen OS, Baillie C, Bryce E, Dawson DA, Ochu E, Hänfling B, Lawson Handley L, Mariani S, Lambin X, Sutherland C, McDevitt AD (2020) Fishing for mammals: Landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems. Journal of Applied Ecology 57: 707–716. https://doi.org/10.1111/1365-2664.13592

Sanches TM, Schreier AD (2020) Optimizing an eDNA protocol for estuarine environments: Balancing sensitivity, cost and time. PLoS ONE 15(5): e0233522. https://doi.org/10.1371/journal.pone.0233522

Sato H, Sogo Y, Doi H, Yamanaka H (2017) Usefulness and limitations of sample pooling for environmental DNA metabarcoding of freshwater fish communities. Scientific Reports 7: 1–12. https://doi.org/10.1038/s41598-017-14978-6

Sato Y, Miya M, Fukunaga T, Sado T, Iwasaki W (2018) MitoFish and MiFish Pipeline: A Mitochondrial Genome Database of Fish with an Analysis Pipeline for Environmental DNA Metabarcoding. Molecular Biology and Evolution 35(6): 1553–1555. https://doi.org/10.1093/molbev/msy074

Seoul Metropolitan Government (2017) The 8th Han River Ecosystem Survey (2nd year). Seoul Metropolitan Government, Seoul. https://lib.seoul.go.kr/search/detail/CATCRZ000001051461?mainLink=/searchS/eaz&briefLink=/searchS/eaz/result?os=-DESC_A_cpp=50_A_sNo=0_A_mainLink=%2FsearchS%2Feaz_A_sq=53_A_st=SUBJ_A_oi=DISP01_A_pn=5# [November 2, 2023]

Shirazi S, Meyer RS, Shapiro B (2021) Revisiting the effect of PCR replication and sequencing depth on biodiversity metrics in environmental DNA metabarcoding. Ecology and Evolution 11(22): 15766–15779. https://doi.org/10.1002/ece3.8239

Shogren AJ, Tank JL, Andruszkiewicz E, Olds B, Mahon AR, Jerde CL, Bolster D (2017) Controls on eDNA movement in streams: Transport, Retention, and Resuspension. Scientific Reports 7: 1–11. https://doi.org/10.1038/s41598-017-05223-1

Smith DP, Peay KG (2014) Sequence Depth, Not PCR Replication, Improves Ecological Inference from Next Generation DNA Sequencing. PLoS ONE 9(2): e90234. https://doi.org/10.1371/journal.pone.0090234

Stewart KA (2019) Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA. Biodiversity and Conservation 28: 983–1001. https://doi.org/10.1007/s10531-019-01709-8

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z (2012) Estimation of Fish Biomass Using Environmental DNA. PLoS ONE 7: e35868. https://doi.org/10.1371/journal.pone.0035868

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E, THOMSEN PF (2012) Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology 21(11): 2565–2573. https://doi.org/10.1111/j.1365-294X.2011.05418.x

U.S. Fish and Wildlife Service (2022) Environmental DNA (eDNA) - Best Management Practices for Project Planning, Deployment, and Application. https://doi.org/10.13140/RG.2.2.12200.96006

Ushio M, Fukuda H, Inoue T, Makoto K, Kishida O, Sato K, Murata K, Nikaido M, Sado T, Sato Y, Takeshita M, Iwasaki W, Yamanaka H, Kondoh M, Miya M (2017) Environmental DNA enables detection of terrestrial mammals from forest pond water. Molecular Ecology Resources 17(6): 63–75. https://doi.org/10.1111/1755-0998.12690

Ushio M, Murata K, Sado T, Nishiumi I, Takeshita M, Iwasaki W, Miya M (2018) Demonstration of the potential of environmental DNA as a tool for the detection of avian species. Scientific Reports 8(1): 1–10. https://doi.org/10.1038/s41598-018-22817-5

Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF, Bellemain E, Besnard A, Coissac E, Boyer F, Gaboriaud C, Jean P, Poulet N, Roset N, Copp GH, Geniez P, Pont D, Argillier C, Baudoin JM, Peroux T, Crivelli AJ, Olivier A, Acqueberge M, Le Brun M, Møller PR, Willerslev E, Dejean T (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Molecular Ecology 25(4): 929–942. https://doi.org/10.1111/mec.13428

Yamamoto S, Masuda R, Sato Y, Sado T, Araki H, Kondoh M, Minamoto T, Miya M (2017) Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. Scientific Reports 7: 1–12. https://doi.org/10.1038/srep40368

Yates MC, Fraser DJ, Derry AM (2019) Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. Environmental DNA 1(1): 5–13. https://doi.org/10.1002/edn3.7

Yates MC, Glaser DM, Post JR, Cristescu ME, Fraser DJ, Derry AM (2021) The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. Molecular Ecology 30(13): 3068–3082. https://doi.org/10.1111/mec.15543

Yeongdeungpo Cultural Foundation (2020) A Study on the Basic Environment of Waterfront Ecological Culture for the Planning of Waterfront Culture in Yeongdeungpo.

Yu Z, Ito SI, Wong MKS, Yoshizawa S, Inoue J, Itoh S, Yukami R, Ishikawa K, Guo C, Ijichi M, Hyodo S (2022) Comparison of species-specific qPCR and metabarcoding methods to detect small pelagic fish distribution from open ocean environmental DNA. PLoS ONE 17(9): e0273670. https://doi.org/10.1371/journal.pone.0273670

Zhang H, Yoshizawa S, Iwasaki W, Xian W (2019) Seasonal Fish Assemblage Structure Using Environmental DNA in the Yangtze Estuary and Its Adjacent Waters. Frontiers in Marine Science 6: 515. https://doi.org/10.3389/fmars.2019.00515

Supplementary material 1

Validation of primers for the detection of eDNA samples

Authors: Yujin Kang, Seungwoo Han, Youngkeun Song

Data type: tif

Explanation note: Genomic DNA was extracted from tissue samples of Eurasian otters at two distinct locations (Tis HD and Tis YY). eDNA samples collected from two sites (HC1 and HC2) at the Korean Otter Research Center were designated as HC (eDNA samples). N Con represented a negative control.

Link: https://doi.org/10.3897/mbmg.8.115512.suppl1

Supplementary material 2

Standard curve with genomic DNA of Eurasian otter

Authors: Yujin Kang, Seungwoo Han, Youngkeun Song

Data type: tif

Link: https://doi.org/10.3897/mbmg.8.115512.suppl2

Supplementary material 3

The physical environment and water quality by seasons of Yeouido Saetgang Ecological Park

Authors: Yujin Kang, Seungwoo Han, Youngkeun Song

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.115512.suppl3

Supplementary material 4

MiSeq sequencing performance statistics of eDNA samples at Yeouido Saetgang in April and October

Authors: Yujin Kang, Seungwoo Han, Youngkeun Song

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.115512.suppl4

Supplementary material 5

Paper reviews about diets of Eurasian otters

Authors: Yujin Kang, Seungwoo Han, Youngkeun Song

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.115512.suppl5

Supplementary material 6

The species observed at each sampling site, along with the number of reads recorded during each sequencing repetition

Authors: Yujin Kang, Seungwoo Han, Youngkeun Song

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.115512.suppl6

Supplementary material 7

The fish fauna detected by eDNA metabarcoding and relative abundance after refining the species which was not recorded in freshwater systems in Korea and natural log transformation

Authors: Yujin Kang, Seungwoo Han, Youngkeun Song

Data type: xlsx

Link: https://doi.org/10.3897/mbmg.8.115512.suppl7